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Investigation 5: Photosynthesis

Background

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen. Like all enzyme-driven reactions, the rate of photosynthesis can be measured by either the disappearance of substrate or the accumulation of product (or by-products).

The general summary equation for photosynthesis is

$$2\ H_2O + CO_2 + \text{light} \rightarrow \text{carbohydrate } (CH_2O) + O_2 + H_2O$$

What could you measure to determine the rate of photosynthesis?

- Production of O_2 (How many moles of O_2 are produced for one mole of sugar synthesized?)

or

- Consumption of CO_2 (How many moles of CO_2 are consumed for every mole of sugar synthesized?)

In this investigation, you will use a system that measures the accumulation of oxygen.

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O_2 and CO_2), leaves — or disks cut from leaves — normally float in water. What would you predict about the density of the leaf disk if the gases are drawn from the spongy mesophyll layer by using a vacuum and replaced with water? How will that affect whether or not the leaf floats? If the leaf disk is placed in a solution with an alternate source of carbon dioxide in the form of bicarbonate ions, then photosynthesis can occur in a sunken leaf disk. As photosynthesis proceeds, oxygen accumulates in the air spaces of the spongy mesophyll, and the leaf disk will once again become buoyant and rise in a column of water. Therefore, the rate of photosynthesis can be indirectly measured by the rate of rise of the leaf disks. However, there's more going on in the leaf than that! You must also remember that cellular respiration is taking place at the same time as photosynthesis in plant leaves. (Remember that plant cells have mitochondria, too!) What else could be going on that might affect this process? Aerobic respiration will consume oxygen that has accumulated in spongy mesophyll. Consequently, the two processes counter each other with respect to the accumulation of oxygen in the air spaces of the spongy mesophyll. So now you have a more robust measurement tool — the buoyancy of the leaf disks is actually an indirect measurement of the *net* rate of photosynthesis occurring in the leaf tissue. A measurement of respiration in the same system would allow the estimation of *gross* production.

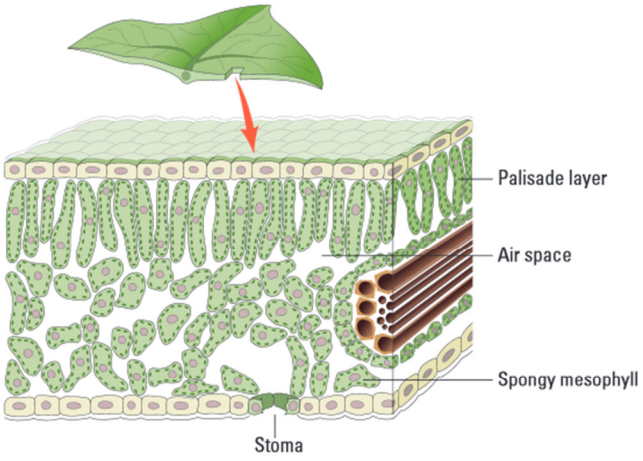

Figure 1. Leaf Anatomy

When immersed in water, oxygen bubbles are

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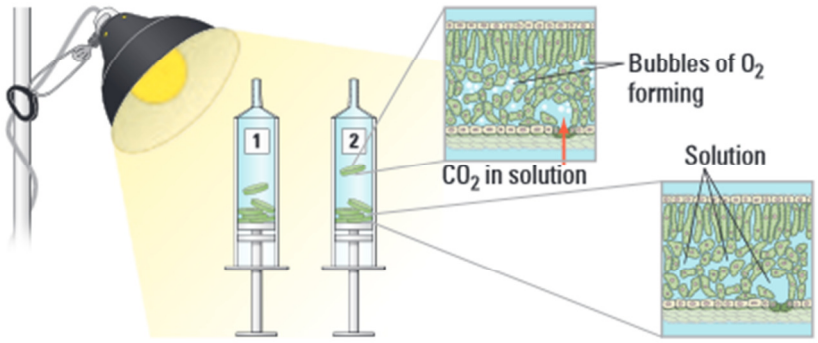
usually trapped in the air spaces of the spongy mesophyll in the plant leaf. By creating a vacuum in this experimental procedure, the air bubbles can be drawn out of the spongy mesophyll, and the space is refilled by the surrounding solution. This allows the leaf disks to sink in the experimental solution. If the solution has bicarbonate ions and enough light, the leaf disk will begin to produce sugars and oxygen through the process of photosynthesis. Oxygen collects in the leaf as photosynthesis progresses, causing the leaf disks to float again. The length of time it takes for leaf disks to float again is a measure of the *net* rate of photosynthesis.



Pre-Lab Questions

1. What is the purpose of creating a vacuum with the floating leaf disks?

2. What is the purpose of the baking soda (sodium bicarbonate)?

3. Why is the floating disk technique performed using leaves and not roots?

4. What causes the disks in the bicarbonate solution to rise after they are placed under a light source?

5. Why is the rate at which the disks float an indirect measurement of the *net* rate of photosynthesis?

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Baseline Procedure

1. Label one syringe "With CO_2" and the other "Without CO_2." Repeat labels on two plastic cups as well.
2. Pour the prepared bicarbonate solution into the "With CO_2" cup to a depth of about 3cm. (The bicarbonate will serve as a source of carbon dioxide for the leaf disks while they are in solution.)
3. Pour deionized water into the "Without CO_2" cup to a depth of about 3cm.
4. Add two drops of a dilute liquid soap to the solution in each cup. It is critical to avoid suds. If either solution generates suds, then dilute it with more bicarbonate or water solution. The soap acts as a surfactant or "wetting agent" – it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and enabling the leaf disks to sink in the fluid.

5. Using a hole punch, cut 10 uniform leaf disks for EACH cup. Avoid major leaf veins.
6. Draw the gases out of the spongy mesophyll tissue and infiltrate the leaves with the sodium bicarbonate solution by performing the following steps:
    a. Remove the plunger from both syringes.
    b. Place 10 leaf disks into the barrel of each syringe.
    c. Carefully replace each plunger, being careful not the crush the leaf disks. Push in each plunger until only a small volume of air and leaf disk remain in each barrel (less than 10% of the volume).
    d. Pull a small volume (3mL) of sodium bicarbonate plus soap solution from your prepared cup into the "With CO_2" syringe. Tap and swirl the syringe to suspend the leaf disks in the solution. Make sure that, with the plunger inverted, the disks are suspended in the solution. Make sure no air remains. Move the plunger to get rid of air from the plunger before you move on.
    e. Repeat step d for the water (transferring water plus soap solution from your "Without CO_2" cup into your "Without CO_2" syringe).
    f. You now want to create a vacuum in each plunger to draw the air out of the leaf tissue. Place the syringe tip cap on each syringe.

    g. Make sure the disks are suspended in solution by lightly shaking each syringe and then draw back on the plungers to create a vacuum. Hold the vacuum for approximately 10 seconds. While holding, swirl the leaf disks to keep them suspended in solution.

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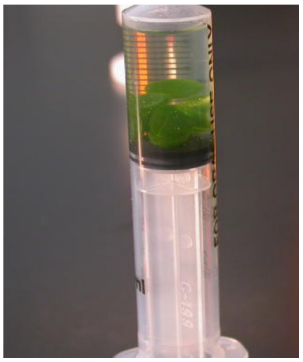
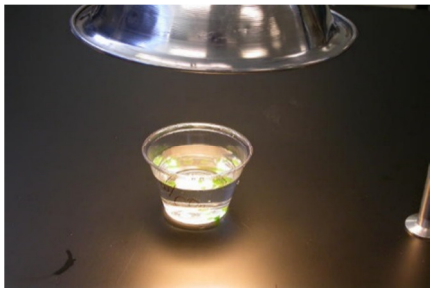
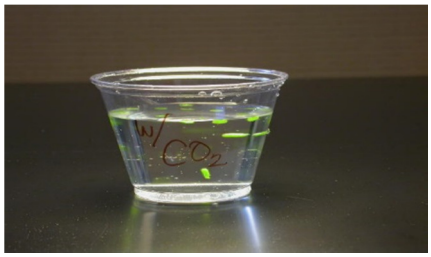
h. Release the vacuum by letting the plunger spring back. The solutions will infiltrate the air pockets in the leaves causing the leaf disks to sink. Repeat step g up to 3 times until all the disks sink.
   - Note: If the disks do not sink after three trials, add a second very small drop of soap to the bicarbonate solution and repeat step 6.
   - Placing the disks under vacuum more than three times can damage the disks.



7. Pour the disks and the solution from each syringe into the appropriate cups.



8. Place both cups under the light source (about 8" away) and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that stuck against the side of the cups. Continue until all of the disks are floating in the cup with the bicarbonate solution.
9. To make comparisons between experiments, a standard point of reference is needed. Repeated testing of this procedure has shown that the point at which 50% of the leaf disks are floating (the median or ET_{50}, the Estimated Time it takes 50% of the disks to float) is a reliable and repeatable point of reference for this procedure.
   Graph your data and calculate your ET_{50}.



Results

Table 1: Without CO_2

Time (min)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
# of floating leaf disks																

Table 2: With CO_2

Time (min)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
# of floating leaf disks																

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Analysis

- Create a graph (scatter plot) measuring the Number of Floating Leaf Disks vs Time using the data from Table 2
  - Independent variable (x-axis): _____
  - Dependent variable (y-axis): _____
- Use your graph to determine the median, or ET_{50}, which is the time required for 50% of the leaf disks to float and record it here: _____
- There is actually an inverse relationship between ET_{50} and the rate of photosynthesis. (ET_{50} goes down as the rate of photosynthesis goes up, which plots a graph with a negative slope.) To create a more traditional direct relationship graph when comparing treatments, calculate the inverse of the ET_{50}, or $1/ET_{50}$ and record it here: _____
- Answer the following questions to reflect on your lab set up and the connection between your results and the process of photosynthesis.
  (Your may also use your notes to help you)

1. What was the control group in this lab and WHY was it necessary?

2. Identify 3 other controls used in this lab (remained constant in experimental and control group)
   -
   -
   -

3. Is oxygen produced in the light-dependent or light-independent reactions?

4. In what cycle is carbon needed in photosynthesis AND where in the chloroplasts does this cycle take place?

5. Identify what product the carbon dioxide is used to make AND explain what is meant by "carbon fixation."

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EXTENSION

In the next part of the lab, you are going to design and carry out an experiment to analyze the effect of the wavelength of light (ie-color of light) on the rate of photosynthesis.

Your Procedure

Write a detailed, reproducible procedure explaining the steps you would take to test AND analyze the effects of red, green, and blue light on the rate of photosynthesis. Use the baseline procedure to help guide you.

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Your Hypothesis

Use your knowledge of photosynthesis and wavelengths of light to predict what color(s) light would produce the fastest vs the slowest rates of photosynthesis.

Your Results

Table 3: Effect of Colored Light on Time for Leaf Disks to Float

Time (min)	# of Floating Leaf Disks		
	Red Light (Wavelength of 622-780 nm)	Green Light (Wavelength of 492-577 nm)	Blue Light (Wavelength of 455-492 nm)
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

Your Analysis

- Create a graph (scatter plot) measuring the Number of Floating Leaf Disks vs Time using the data from Table 3
  - Be sure to
    - Include a title for your graph
    - Label each axis
    - Number each axis with an appropriate scale
    - Make a key to distinguish between the 3 different colors of light used

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- Use your graph to determine the median, or ET_{50}, which is the time required for 50% of the leaf disks to float under each color, and record them below in Table 4
- To more accurately compare the effect of the wavelength (ie-color) of light on the rate of photosynthesis, calculate the inverse of the ET_{50}, or $1/ET_{50}$ for each light color/wavelength and record them below in Table 4

Table 4: Rates of Photosynthesis under Different Wavelengths of Light

Color of Light	Wavelength of Light	ET_{50}	$1/ET_{50}$
Red	622-780 nm		
Green	492-577 nm		
Blue	455-492 nm		

- Use your $1/ET_{50}$ from Table 4 to create a bar graph showing the effect of wavelength of light on rate of photosynthesis
    1. Independent variable (x-axis): Wavelength of Light (nm)
        - You can also indicate color
    2. Dependent variable (y-axis): Rate of Photosynthesis ($1/ET_{50}$)

- Answer the following questions to reflect on your lab set up and the connection between your results and the process of photosynthesis.
    1. Explain at least one source of error that could have influenced your data in this part of the lab.

    2. Suppose after the leaf disks floated, we turned off all of the lights. Predict what would happen to the number of floating disks and WHY.

    3. Other than changing the wavelength/color of light used in the experiment, explain at least one other variable you could investigate using the floating leaf disk technique to determine its effect on the rate of photosynthesis.

Your Conclusion

- Explain which wavelengths/colors of light resulted in the fastest AND slowest rates of photosynthesis and WHY.